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# Sensitive Electro-chemical Determination of Antidepressant drug Clomipramine at f-MWCNTs Nano Clusters modified Glassy Carbon Electrode

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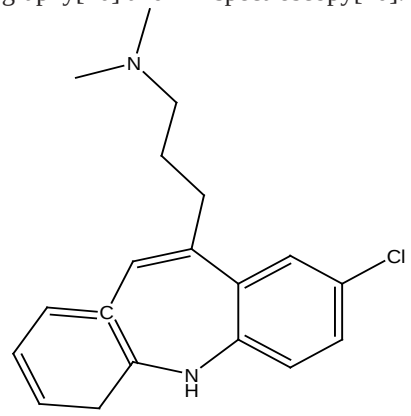
**Abstract**:

Clomipramine, an important tricyclic antidepressant drug with low redox activity, was effectively electro catalyzed on Functionalized nano-clusters modified glassy carbon electrode (i.e.,f-MWCNTs/ GCE) and generated a sensitive anodic peak at about 1.120 V in pH 5.5. Furthermore, determination of clomipramine in bulk form and human urine as a biological sample was carried out by differential pulse anodic adsorptive stripping voltammetry using optimized parameters. The resulting sensor exhibited a considerable enhancement in voltammetry response characteristics: extending the linear range and lowering the detection limit. The proposed differential pulse stripping voltammetric method shows linearity over the concentration range 1.45 x $10^{-5}$-4.52 x $10^{-3}$M. The achieved limits of detection (LOD) and quantification (LOQ) 1.315 x $10^{-8}$g/mL and 2.83 x $10^{-8}$g/mL. This method was successfully applied to the determination of clomipramine in drug tablets and proved to be reliable compared with UV.

**Keywords:** Clomipramine Hydrochloride, cyclic voltammetry, stripping Voltammetry, Pharmaceuticals, Glassy Carbon Electrode, Carbon Nanotubes

## 1. INTRODUCTION

As a very important tricyclic antidepressant drug, clomipramine (Scheme 1,C19H23ClN2) is widely used in the form of its hydrochloride to treat anxiety and obsessive-compulsive disorder[1-4]. Generally, the determination of clomipramine is performed with spectrophotometry (Lima et al. 2002), chemiluminescence (Marques et al. 2004), chromatography (Nevado et al. 2005), capillary zone electrophoresis (Kou et al. 2004), and fluorometry (Mohamed et al.2005), and so on. Nevertheless, there are few reports about clomipramine studied by electrochemical methods for its low redox activity under normal conditions. Bishop and Hussein studied clomipramine electrochemical behavior using rotating gold and platinum electrodes (Bioship and Hussein 1984).Ivandini et al. (2002) used highly boron-doped diamond electrodes by HPLC to detect it and got satisfactory results, but the preparation of the electrodes were complicated and time–consuming. Ortuno et al. (2006) fabricated a novel ion-selective electrode for clomipramine detection, the electrode had high stability and rapid respondence. Recently, we fabricated a 16-mercaptohexadecanoic acid self-assembled monolayer modified gold electrode and modified electrode presented high electrocatalytical activities toward clomipramine (Huang et al. 2007) [5-7]. Metal nanoparticles are very increasingly used for the electrode modification owing to their extraordinary catalytic activities over corresponding bulk metal electrodes (El-Deab and Ohsaka 2003). Pt nanoparticles have been intensively studied, especially for the subject of fuel cell constructions (Tong et al. 2000). Lin's group synthesized Pt/Fe(III) and Pt/MWNT nanocomposite clusters directly on the electrode surface, and these modified electrodes presented high electrocatalytical activities toward some substances(Wang et al. 2004; Lin and Li 2006) [8-10]. This composite effect between different nanostructures is obviously interesting for obtaining high efficient biosensors. In the past decades, polymer modified electrodes have attracted great attention, as polymer films have good stability, reproducibility and more active sites (Volkov et al. 1980; Ohnuki et al. 1983)[11-13]. Electropolymerization is a good approach to immobilize polymers to prepare modified electrode as adjusting the electrochemical parameters can control film thickness, permeation and charge transport characteristics. Glassy carbon electrodes (GCEs) have been widely used compared with metal electrodes because of its biocompatibility with tissue, having low residual current over a wide potential range and minimal propensity to show a deteriorated response as a result of electrode fouling (Ewing et al. 1981; Lane and Blaha 1990; Mattson and Jones 1976). In this paper, a novel nanocomposite material-MWCNTs was fabricated for determination of clomipramine.[14-15] There have been few reported analytical methods for the estimation of clomipramine viz., high performance liquid chromatography[17], mass spectrometry[18], $^{1}$H-NMR spectroscopy[17], gas liquid chromatography[20] and IR- spectroscopy[20].




Scheme: 1 The chemical structure of clomipramine

## 2. EXPERIMENTAL

2.1 Chemicals and reagents

All chemical used were of analytical reagent grade quality and emplode without further purification. Multiwalled carbon nanotube with a96%purity,o.d.=9-18 nm and 0.4-48µm tube length were obtained from Aldrich. Clomipramine Hydrochloride (98% pure) was obtained from local market under the trade name Clonil and was used without further purification stock standard solution of bulk clomipramine hydrochloride ($4x10^{-3}$molL-1) was prepared in water and stored at 25°C until assay. Tablets of clomipramine hydrochloride was weight and ground to a homogeneous fine powder in a mortar portion of the finely ground material equivalent to 25 mg of Clomipramine Hydrochloride accurately weight and transferred into a 10 ml calibrated flask containing 50 ml water. The working solutions ($4 \times 10^{-6}$ to $3 \times 10^{-3}$mpl L-1) were prepared daily by appropriate dilution of the standard solution of bulk clomipramine hydrochloride with water just before use. A series Britton-Robinson buffer (BR-buffer) of pH values 2 to 12 was prepared and KCl used as a supporting electrolyte.

2.2 Apparatus

All electrochemical measurements were performed using a model 1230A [SR 400] electrochemical analyzer (CHI Instrument, USA). Controlled potential coulometric experiments were carried out on model 760 electrochemical workstation (CHI Instrument). A three electrode cell system incorporating the glassy carbon electrode as working electrode, platinum wire as an auxiliary electrode and Ag/AgCl reference electrode along with temperature controlling support was used throughout the experiment and all experiments were carried out at standard temperature of 25oc. All pH-metric measurements were made on a CHINO digital pH meter fitted with a glass electrode standardized with buffers of known pH.

2.3 Anayltical proparation

2.2.1 Pharmaceutical preparation

clomipramine tablets were used as pharmaceutical dosage form, purchased from local market, each tablet containing 20mg of clomipramine Hydrochloride along with some excipients. To prepare the solutions of tablets, initially the drug content of ten tablets was weighed and finely powdered. The average mass per tablet was determined. A portion of the finely grounded material equivalent to 11.75mg of clomipramine Hydrochloride was accurately weighed and dissolved in 20ml of Methanol; further the solution was diluted with 15ml of water and subjected to sonication for 15 minutes in order to get homogenous solution. After dissolution, content of the flask was centrifuged for 30 min at 1500 rpm. An aliquot of $4\times10^{-3}$mol/L of the solution was then analyzed according to the proposed voltammetric procedure after diluting its appropriate volume with 9ml of BR buffer in electrochemical cell and performed determination of Clomipramine Hydrochloride in tablets by using calibration curve method.

The effective concentration (E.C.) of the sample in the electrochemical cell was calculated as (Concentration of analyte solution × Volume of analyte solution added in the cell)/Total volume of solution in the cell. Where concentration of solution is measured in mol/L and volume of the solution is measured in mL. The concentration mentioned throughout this research work is in terms of effective concentrations. Pretreatment of Glassy Carbon Electrode and Voltammetric procedure: The working electrode GCE was polished with 0.08m Alumina slurry and further subjected to sonication for a short

duration prior to each measurement in order to remove all impurities remained onto the surface of the electrode and dried at 30o C in Oven. While for the deoxygenation of sample solutions, a continuous stream of Nitrogen gas (99% purity) was allowed to pass through the solutions before each of the voltammetric measurements.

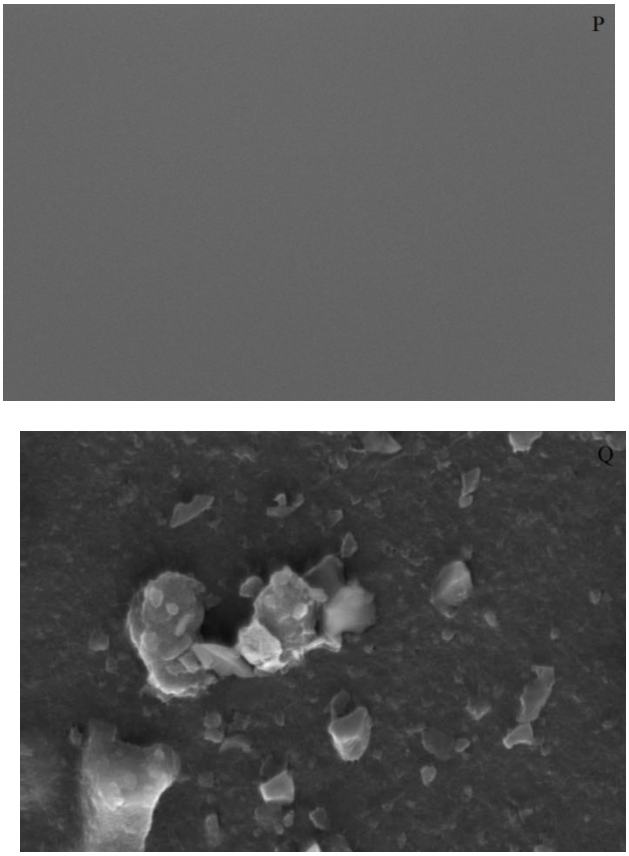



Figure-1: SEM image (P) Bare/GCE (Q) f-MWCNTs-Nafion modified surface of GCE

2.3 Preparation of MWCNTs suspension, and modification of glassy carbon electrode

4 mg of MWCNTs were acidified with 4ml of a mixture of acid ($HNO_3$ : $H_2SO_4$) in ratio of 1:3 respectively for 2h and acidified CNTs were washed with water until water gave pH 7.0. These activated CNTs were dried and then dispersed in the mixture of N,N- dimethylformamide (2mL) + 0.5 ml Nafion [ A conducting polymer ( 0.5% in ethonal) ] and diluted with water followed by hot sonication for 4 hr in an ultrasonic bath to get homogeneous suspension. 10µL of this suspension was allowed to drop onto the pre-treated bare glassy carbon electrode surface using a micropipette and left to dry at room temperature.

2.4 Morphology and Surface characterization of MWCNTs-Nafion/ GCE

Scanning electron micrographs'(SEM) were used for the morphological studies of MWCNTs modified surface of GCE using ZEISS-EV018 instrument from University Science and Instrumentation Centre (University of Rajasthan, Jaipur). The CNTs can be seen in the Scanning electron micrograph covering the surface area of bare Glassy carbon electrode (figure 1). The doping of the bare GCE resulted into grate improvement in the peak response due to the increased surface area and better electron transfer rate between electrode and electrolyte interface. It is well documented that the modification of electrode surface by the MWCNTs increase anodic peak current $I_p$ can be calculated by the expression.[21].

$$Ip = (2.69\times10^5)n^{3/2}A\ Co\ Do^{1/2}\ \nu^{1/2} \quad (1)$$

For $K_4Fe(CN)_6$, n=1 and D= $7.6x10^{-6}$.From the slope of the Ip versus $\nu^{1/2}$ relation, the microscopic areas are found to be 0.0443 $cm^2$ for the bare GCE and 0.0872 $cm^2$ for the MWCNTs-GCE electrodes[22]. Evidently, the modified electrode had an increased surface area of nearly 70%.

## 3. Results And Discussion

3.1 Effect of pH

The effect of different supporting buffers (B-R, acetate, and citrate and phosphate buffer) on the current response of clomipramine hydrochloride is studied in order to assess their impact on the monitored electro analytical signal. The best results with respect to sensitivity accompanied with sharper response were obtained with BR-buffers. Thus study was made in BR-buffers of pH range 2.0 to12.0 at a target concentration of 3.6 X $10^{-4}$ g/mL aqueous clomipramine hydrochloride solution (Fig. 1A). The relation between Ep of the wave and pH of the medium over the range 2.0–12.0 may be expressed by the following expressions:

DPV : Ep (V) (vs. Ag/AgCl) = 0.0551 pH + 0.992; $r^2$ = 0.9928 (2)

DPSV: Ep (V) (vs. Ag/AgCl) = 0.0575 pH + 1.032; $r^2$ = 0.9964 (3)

With the rise in pH the peak potential shifted towards more negative potential which indicated the prior protonation of clomipramine hydrochloride. The height of the peak reaches maximum at pH 5.5. Therefore, pH 5.5 was chosen as the optimum one for the determination of clomipramine hydrochloride. The slope was 57.5 mV per pH,which is close to the theoretical value of 59 mV per pH ,this indicate that the deprotonation step of clomipramine is prior to the electron transfer step and that the number of protons and transferred electrons involved in the oxidation' mechanism is equal[23].

3.2. Effect of scan rate

The effect of scan rate ($\nu^{1/2}$) on peak current (Ip) was examined under the above experimental conditions. As the sweep rate is increased from 10 to 100 mV/s at a fixed concentration of clomipramine, the peak potential shifted towards a more positive value with increase in current confirming the irreversible nature of the oxidation process [23-24].

On subsequent scans (i) the peak potential shift anodically and (ii) the peak current function, Ip(μA)/ $\nu^{1/2}$exhibits almost constancy, where A is cross sectional area of electrode in $cm^2$, C is concentration of clomipramine in g/mL. When peak current (Ip) was plotted against square root of scan rate ($\nu^{1/2}$) a straight line was obtained following the Randles-Sevcik equation.

$$Ip = (2.99\times10^5)n[n']^{1/2}\ A\ Co\ Do^{1/2}\ \nu^{1/2} \quad (4)$$

Where n is the number of electrons exchanged in reduction, n' is the number of electrons involved in the rate determining step of the electrode process, α is the charge transfer coefficient, A($cm^2$) is cross sectional area of the electrode, Co ($mol/cm^3$) is the concentration of the electroactive species in the bulk solution, Ip(A) is the anodic peak current, Do($cm^2\ s^{-1}$) is the diffusion coefficient of the electro active species being oxidized and ν (Vs-1) is the scan rate [8].

A linear Randles–Seveik plot (plot of Ip vs. $\nu^{1/2}$) is obtained indicating that the diffusion is the means of mass transport [9] .

$$Ip\ (\mu A) = 0.009\ \nu^{1/2}\ (mV/s) + 0.994;\ r^2 = 0.998 \quad (5)$$

The finding was further confirmed by plotting log Ip vs. log ν; a straight line was observed which can be expressed by the equation:

$$\log Ip\ (\mu A) = 0.455 - 0.026 \log \nu(mV/s);\ r^2 = 0.992 \quad (6)$$

The obtained slope of 0.45 is close to 0.5 also confirms diffusion controlled nature of the electrode process[25].

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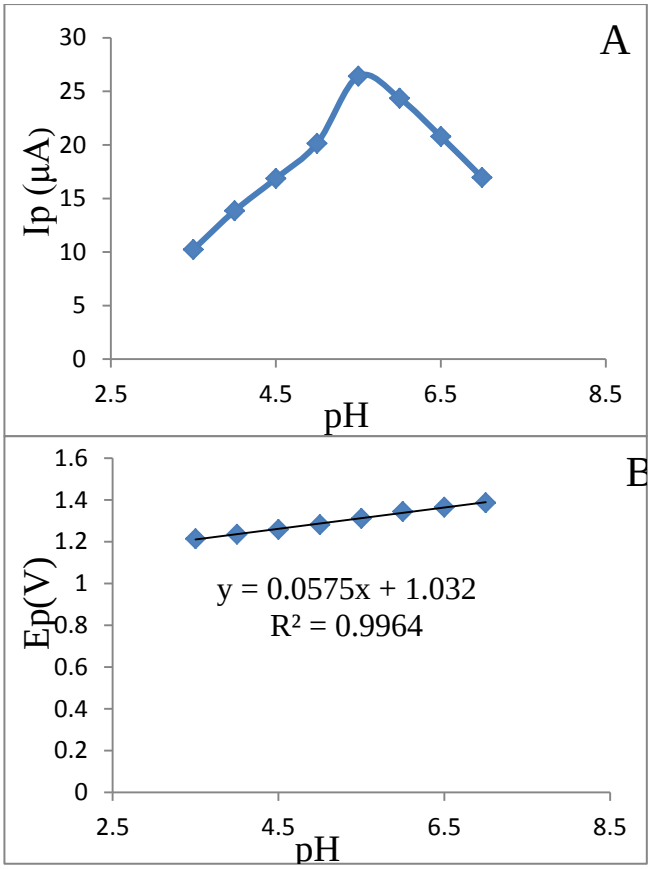



Figure-2: Influence of pH on DP-AAdCV peak current of 3.6 x $10^{-4}$mol $L^{-1}$ Clomipramine in BR-Buffer (pH 3.5-7.0) (Figure A) and plot of the peak potential (Ep ) versus pH (Figure B )

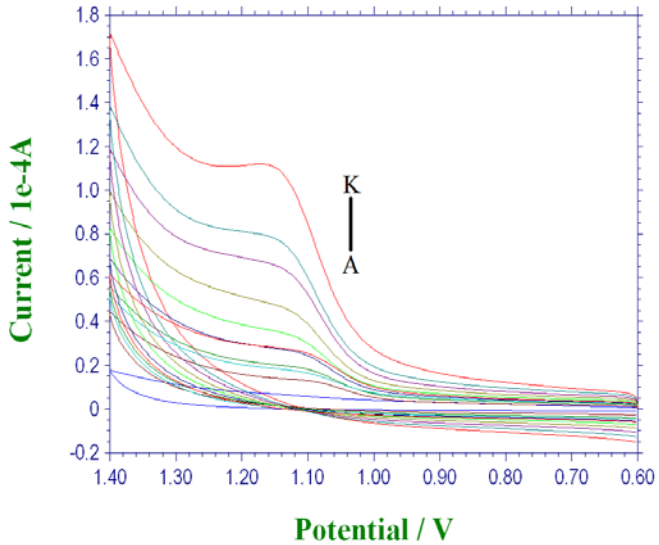



Figure-3: (B) Cyclic Voltammogram of Clomipramine at different scan rates with MWCNT (A)blank (B)20mV/s (C)40mV/s (D)60mV/s (E)80mV/s (F)100mV/s v (G)120mV/s (H)140mV/s (I)160mV/s (J)180mV/s (K)200mV/s at pH 5.5 in BR-Buffer (Concentration 3.6 × $10^{-4}$M).

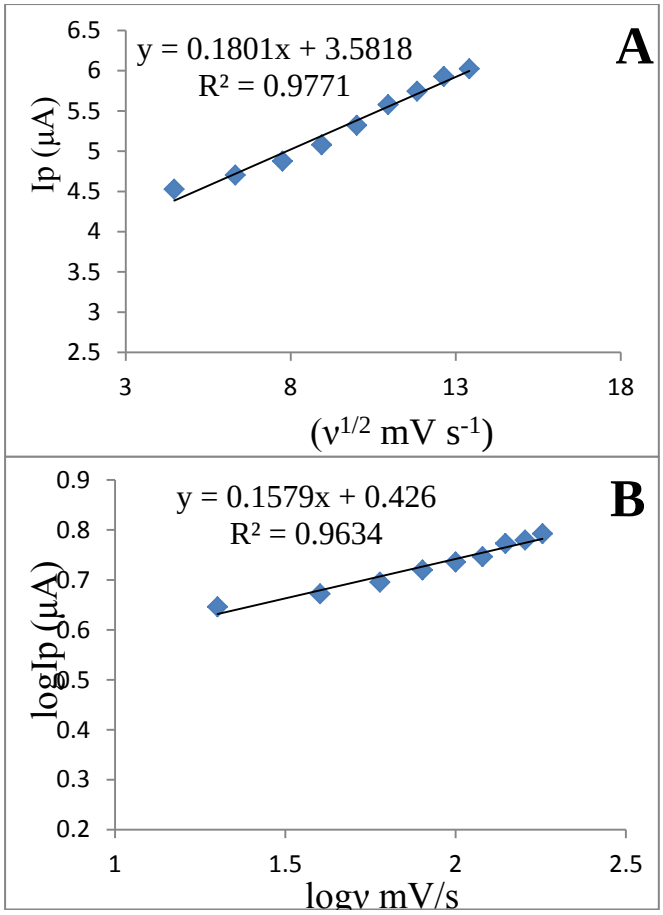



Figure-4: Plot of peak current (Ip) versus square root of scan rate ($v^{1/2}$) from voltammogram in figure-3 for Clomipramine Hydrochloride in 3.6×$10^{-4}$M concentration in BR- Buffer of pH 5.5 ( Figure **A**) and Plot of logarithm peak current (logIp) versus logarithm of scan rate (logv) (Figure **B**)

### 4. Kinetics Of Oidation Of Clomipramine

4.1 Determination of parameter [αn'] and heterogeneous electrochemical rate constant ($k_o$)

According to Laviron's theory, the Ep is defined by the following equation [25]:

$$E = E_0+(2.303RT/ \alpha n' F) \log (RTk_0/\alpha n' F)+(2.303RT/ \alpha n' F) \log v \quad (9)$$

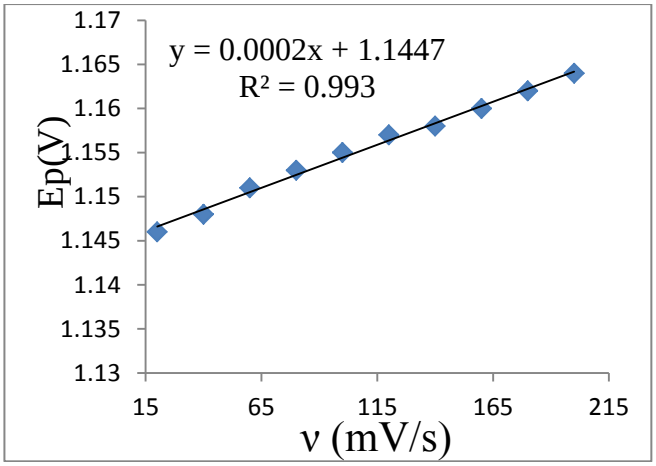
Where α is the transfer coefficient, ν the scan rate, n the number of electron transferred, $k_0$ the standard heterogeneous rate constant of the reaction and $E_0$ is the formal redox potential. And R, T and F have their usual meanings. Thus αn' was easily calculated to be 1.078 from the slope of Ep versus log ν.

Straight line of Ep *vs.* logν plot is expressed by the following linear regression equation:

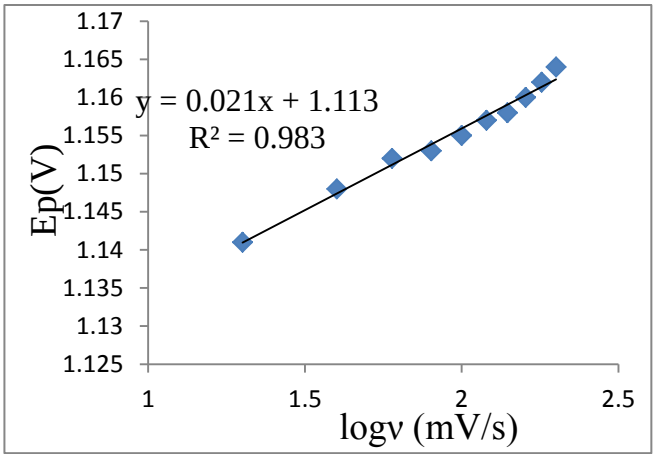
$$Ep = 0.021 \log v + 1.113 \quad (10)$$

$r^2 = 0.983$

The value of αn' was calculated by comparing slope of equations 5 and 6 and was found equal to 1.0789.




A




B

Figure-5: Plot of peak potential (Ep) versus scan rate (ν) from voltammogram in figure-3 for Clomipramine in $3.6\times10^{-4}$M concentration in BR- Buffer of pH 5.5 ( Figure **B**) and Plot of peak potential (Ep) versus logarithm of scan rate (logν) (Figure **A)**

4.2 Determination of total number of electrons

The total number of electrons (n) involved in overall oxidation process was calculated by analyzing the charge consumed by desired concentration of clomipramine. This was accomplished by taking 5 mL of 4 mg $mL^{-1}$ solution of clomipramine in a cell and electrolysis was performed at a potential of 1.121 against Ag/AgCl reference electrode for10 hours. During the electrolysis, solutions were kept stirred and purged with nitrogen. Due to long-time electrolysis, current efficiency and completion of electrolysis were assumed to be nearly 100% and 99.98% respectively. The total number of electrons (n) involved in overall oxidation process was calculated using the formula Q = nFN, where Q is charge in coulombs, N is number of moles of clomipramine and F is Faraday's constant. The value of n was found to be 2 for clomipramine at f-MWCNTs/GCE[28].

4.3 Determination of diffusion coefficient (Do $cm^2/s$)

Electro-oxidation of $2.7\times10^{-4}$ M clomipramine at the GCE and NAF-CNT-GCE was investigated by employing chronocoulometry for the determination of the kinetics and mechanisms of electrode reactions. Employing double-potential step chronocoulometry, after point-by-point background subtraction, the plot of charge (Q) vs. the square root of time ($t^{1/2}$) showed a linear relationship. According to the integrated Cottrell equation, the diffusion coefficient and Qads of clomipramine could then be estimated from the slope and intercept, respectively, of the plot of total Q vs. $t^{1/2}$, given by the Anson equation [34]. The resulting calculated parameters are presented in Table-2. As can be seen from the table, the value of the slope and the Qads for the f-MWCNTs/GCE were more than that for other electrodes, confirming that NAF along with CNTs makes the accumulation of clomipramine onto the electrode surface more effective.

The surface coverage ($\Gamma^{o}$) for all four electrodes was calculated using the following relationship:

$$Q_{ads} = nFA\Gamma^{o} \qquad (11)$$

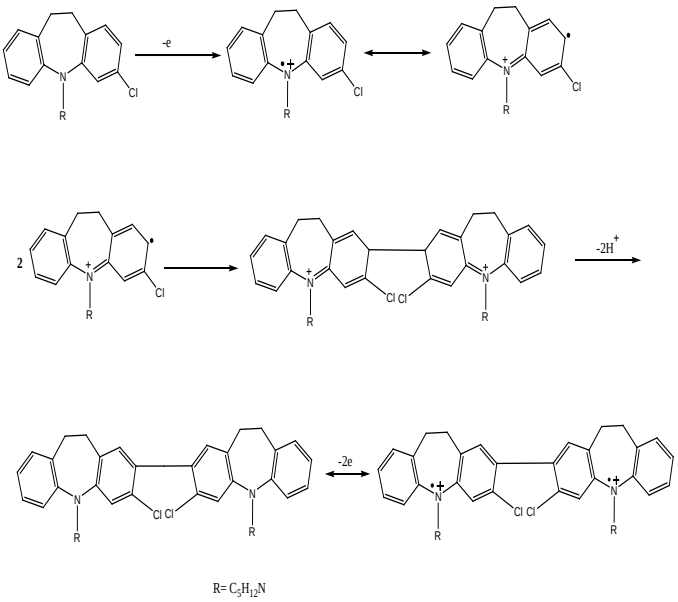
and the results are given in Table-2. From these values, it was observed that the surface coverage was maximum in the case of the NAF-CNT-GCE. Thus, due to the synergistic effect of NAF and CNTs,
the electrode surface coverage by clomipramine drastically increased and the kinetics of oxidation became more facile, confirming the results obtained from CV[34].

$$Q_d = \frac{2nFAD_O^{\frac{1}{2}}C_O t^{\frac{1}{2}}}{\pi^{1}/_{2}} \qquad (12)$$

Integrated Cottrell equation

## 5. Proposed Oidation Mechanism

On the basis of $P^H$, CV and CPC studies, it was concluded that 2 electrons and 2 protons were participating in the oxidation process of clomipramine [26-27]. A oxidation mechanism was proposed based on all experimental observations (Scheme 2):




**Scheme 2.** Proposed oxidation mechanism of Clomipramine

## 6. Electroanalytical Determination Of Clomipramine

Since voltammetric methods have cost-effectiveness high accuracy, precision, sensitivity and absence of lengthy extraction processes, therefore, they are widely used for analytical purposes. In the present paper, differential pulse voltammetry and differential pulse anodic adsorptive stripping voltammetric technique was optimized for the determination of Clomipramine in bulk form and in human urine at GCE.

## 7. Optimization Of Parameters

Operational parameters such as accumulation time ($t_{acc}$), accumulation potential ($E_{acc}$), scan increment ($\Delta S$), peak to peak amplitude, pulse amplitude ($E_{sw}$), pulse period and pulse width etc. were optimized before recording DP-AAdS voltammograms to get best response in terms of peak shape, peak current, peak height and peak stability. The optimized parameters are given in Table 1.

**Table 1.** The optimized experimental parameters of DP-AAdSV procedure

| Optimized operational parameters for DP-AAdSV | Optimized operational parameters for DPV |
|---|---|
| Scan increment (mV) 04 | Scan increment(mV) 05 |
| Pulse amplitude (mV) 50 | Pulse amplitude(mV) 50 |
| Deposition time (s) 15 | Deposition time (s) 16 |
| Deposition potential (V) 0.0 | Deposition potential (V) 0.0 |
| Pulse width (s) 0.2 | Pulse width (s) 0.3 |
| Pulse period (s) 0.5 | Pulse period (s) 0.4 |

## 8. Effect Of Concentration

In order to determine the effect of concentration of clomipramine hydrochloride on DPV and DP-AAdSV peak current, voltammograms of clomipramine are recorded at f-MWCNTs/GCE. The linearity evaluated by linear regression analysis was calculated by least square regression method[28-30]. The calibration curve constructed for clomipramine hydrochloride is linear over the concentration $2.1\times10^{-5}$ to $1.4\times10^{-4}$M for DPV 1.45 x $10^{-5}$-4.52 x $10^{-3}$M for DP-AAdSV method. Since the differential pulse anodic adsorptive stripping voltammetry (DP-AAdSV) is more sensitive than differential pulse anodic adsorptive voltammetry, detailed studies are carried out using differential pulse anodic adsorptive stripping voltammetry. The calibration curves were represented by the following equations:

DPV: $I_p$ (μA) = (117.19) C (M) + (6.4927); $r^2 = 0.9837$; n = 2 (13)

DP-AAdSV: $I_p$ (μA) = (137.59) C (M) + (4.6138); $r^2 = 0.9915$; n = 2 (14)

The regression plots showed that there was a linear dependence of the current intensity on the concentration in both DPV and DP-AAdSV modes over the range given in Table 3.

### 8.1. Detection and quantification limit

Detection limit is calculated by equation LOD= 3 S.D. /b, where S.D. is standard deviation of intercept and b is slope of the regression line. The calculated LOD values of clomipramine are $1.315\times10^{-8}$ g/mL for DP-AAdSV and $4.37\times10^{-7}$ g/mL for DPV method.

The quantitation limit (LOQ) is examined by the equation LOQ= 10 S.D. /b. These values are $2.83\times10^{-8}$ g/mL for DP-AAdSV and $8.78\times10^{-7}$ g/mL for DPV method.

### 8.2. Accuracy and precision

The intra-day and inter-day accuracy and precision of the proposed procedure was estimated by analyzing 2.34-5.13 g clomipramine solutions for three times in four successive days (Table 4). The relative error of 0.30 to 2.10 and %RSD of 1.35–1.62 indicates the high accuracy and precision of the proposed method [31-32].

## 9. Application Of Analytical Determination To Spiked Human Serum And Urine Samples

The significance of the proposed method was examined by employing it for the determination of clomipramine in spiked serum and urine as a biological sample [33]. Without any requirement of time-consuming extraction or evaporation step for sample preparation the proposed method can be applied simply after dilution step with direct measurements in acidic media. Results of the analytical study of spiked serum are summarized in Table 4 & 5.

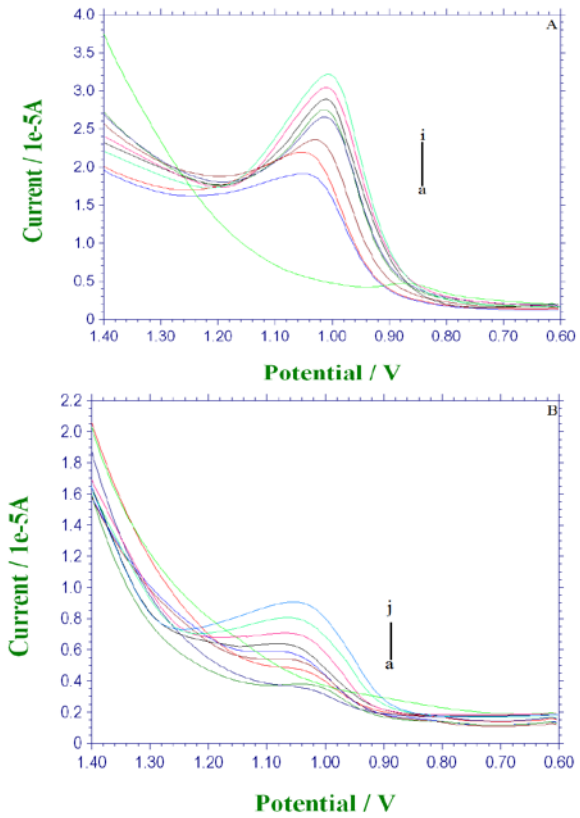



Figure-6: Figure(A) The dependence of the differential pulse anodic adsorptive voltammgram peak current(Ip) of clomipramine of different concentrations in $3.6\times10^{-4}$ mol/L at MWCNTs-GCE; pH 5.5 (a) blank, (b) $0.8\times10^{-4}$g/mL, (c) $1.0\times10^{-4}$g/mL, (d) $1.2\times10^{-4}$ g/mL, (e) $1.4\times10^{-4}$ g/mL, (e) $1.6\times10^{-4}$ g/mL, (g) $1.8\times10^{-4}$g/mL, (h) $1.8\times10^{-4}$g/mL and (i)$2.0\times10^{-4}$ g/mL. and Figure (B) The dependence of the differential pulse anodic adsorptive stripping voltammgram peak current(Ip) of clomipramine of different concentrations in $3.6\times10^{-4}$ mol/L tetra methyl ammonium bromide at MWCNTs-GCE; pH 5.5 (a) blank, (b) $0.6\times10^{-4}$g/mL, (c) $0.8\times10^{-4}$g/mL, (d) $1.0\times10^{-4}$ g/mL, (e) $1.2\times10^{-4}$ g/mL, (e) $1.4\times10^{-4}$ g/mL, (g) $1.6\times10^{-4}$g/mL, (h) $1.8\times10^{-4}$g/mL and (i)$2.0\times10^{-4}$ g/ mL

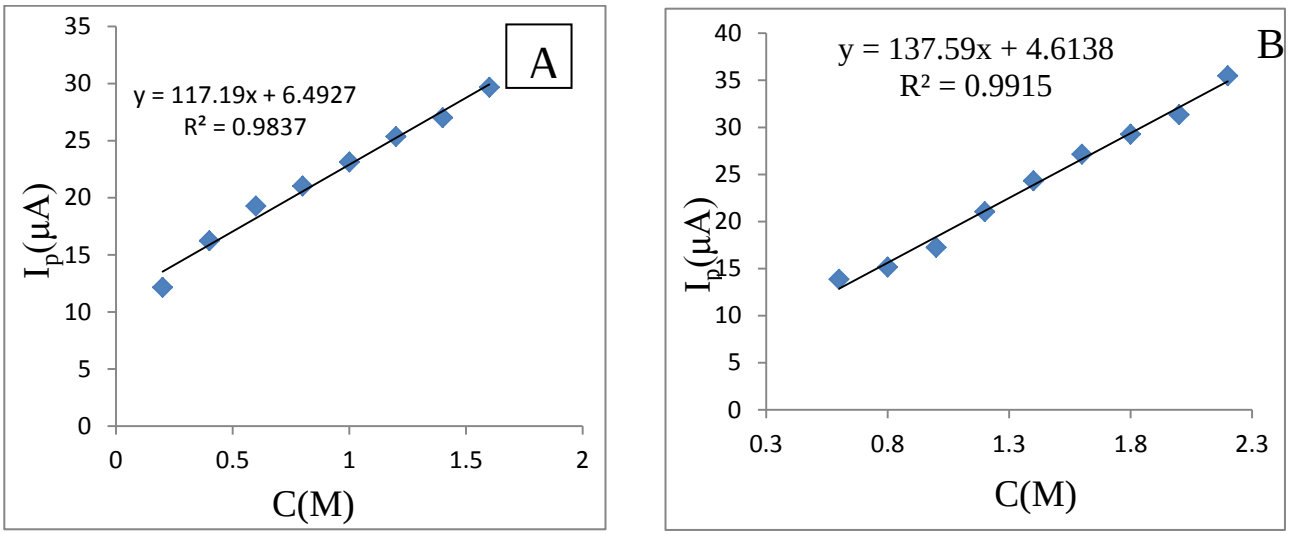



Figure-7:(A) plot peak current ($I_p$) versus C(M) in DPV and figure (B) peak current ($I_p$) versus C(M) in DP-AAdSV

**Table-2** Comparable electrochemical behavior of $2.7x10^{-4}$ g/mL Clomipramine determined at bare-GCE and MWCNTs-GCE

| Electrolyte( buffer pH 6.5) | $E_p$/V (Ag/AgCl) | $I_p$/μA | $D_o$ $cm^2/s$ | $k_o$ ($s^{-1}$) | Γ$^o$ |
|---|---|---|---|---|---|
| Clomipramine in bare-GCE | 1.12 | 2.652 | $2.32x10^{-4}$ | 0.767 | $4.23 \times 10^{-3}$ |
| Clomipramine in MWCNTS-GCE | 0.98 | 30.34 | $6.45x10^{-4}$ | 1.241 | $98.01 \times 10^{-3}$ |

**Table 3** Analytical parameters for voltammetric determination of clomipramine using DP-AAdSV and DPV.

| **Modified method** | Con.range | Regression equation(Ip vs.Con.) | $r^2$ | LOD | LOQ | S.D. |
|---|---|---|---|---|---|---|
| DPV | $2.1 \times 10^{-5}$ -1.4 x $10^{-3}$ M | $I_p$ (μA) = (117.19) C (M) + (6.4927 | 0.9837 | $4.37 \times 10^{-7}$g/mL | $1.8.78 \times 10^{-7}$g/mL | $1.66x\ 10^{-4}$ |
| DP-AAdSV | $1.45 \times 10^{-5}$-4.52 x $10^{-3}$M | $I_p$ (μA) = (137.59) C (M) +4.6138 | 0.9915 | $1.315 \times 10^{-8}$g/mL | $2.83x\ 10^{-8}$g/mL | $3.89 \times 10^{-4}$ |

.

**Table 4.** Recovery results of proposed method for spiked human urine samples (solution of standard clomipramine was spiked)

| Sample | Amount added, mg/L | Amount found, mg/L | Recovery[a] | %RSD |
|---|---|---|---|---|
| Standard in serum sample | 10 | 9.75<br>9.34<br>9.98<br>10.0<br>9.65 | 8.97±0.34<br>$t_{cal}$ = 2.03<br>$t_{tab}$ = 1.78 | 1.35 |

[a]*Results of recovery values are given as mean ± ts/_n (at 94% confidence level)*

**Table 5.** Recovery results of proposed method for spiked human serum samples (solution of Clomipramine was spiked)

| Sample | Amount added, mg/L | Amount found, mg/L | Recovery[a] | %RSD |
|---|---|---|---|---|
| Standard in serum sample | 10 | 9.98<br>9.75<br>9.53<br>10.0<br>9.85 | 9.97±0.51<br>$t_{cal}$ = 1.785<br>$t_{tab}$ = 2.45 | 1.62 |

[a]*Results of recovery values are given as mean ± ts/_n (at 94% confidence level)*

## 10. CONCLUSION

Electrochemical behavior of antidepressant medication clomipramine was studied at bare and modified glassy carbon electrode, using CV, DPV and DP-AAdSV technique, in bulk formulation. It was found that oxidation process of clomipramine was irreversible, diffusion controlled and pH dependent. Furthermore, kinetic parameters such as diffusion coefficient (Do), number of electrons ($n'$) and electron transfer coefficient ($k_0$) were also calculated which were used to propose oxidation mechanism. DP-AAdSV and DPV method was employed for the determination of clomipramine in a blood sample as a biological sample. The proposed method is direct, simple, and cost-effective, requires only small amount of analyte and does not involve tedious steps such as separation, filtration, extraction, and evaporation etc., required by chromatographic methods. Furthermore, the proposed method has good operational characteristics such as extremely low values of detection limits, sensitivity, selectivity, wide liner working range and exhibit good accuracy, precision and repeatability for determination of clomipramine in bulk as well as in serum and urine as a biological sample.

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